Technical Bulletin

3’ to 5’ Exonuclease Activity Assay (Fluorometric)

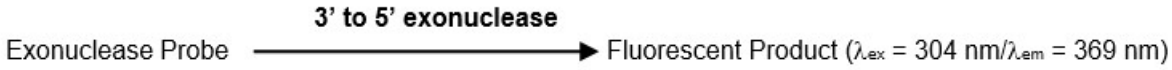
Catalog Number MAK416

Product Description

Exonucleases remove a single nucleotide or an oligonucleotide from the 5’ or 3’ end of a longer oligonucleotide. Exonuclease, such as Three Prime Repair Exonuclease 1 (TREX1), plays an important role in proofreading DNA amplification and removal of damaged DNA. Deficiency of TREX1 enzyme has been shown to trigger the autoimmune disease Aicardia-Goutieres syndrome. TREX1 expression has been reported to correlate with cervical cancer cell growth and disease progression. In addition, 3’-exonucleases are often used as tools in molecular biology such as site-directed mutagenesis and the generation of single stranded DNA probe.

The 3’ to 5’ Exonuclease Activity Assay kit provides a quick and easy method for monitoring 3’-exonuclease activities in a wide variety of samples. In this assay, exonuclease digests the provided DNA probe, producing a strong fluorescent signal (λ_{Ex} = 304 nm/λ_{Em} = 369 nm). The assay is simple, sensitive, and adaptable to high-throughput applications. The method can detect as little as 0.2 μU of exonuclease activity.

The kit is suitable for the measurement of 3’ to 5’ exonuclease activity in biological samples (e.g., mammalian and bacterial cell culture and animal tissue) and for quality control of purified 3’ to 5’ exonuclease.



Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Exonuclease Assay Buffer 25 mL
  Catalog Number MAK416A
- Exonuclease Probe 1 vial
  Catalog Number MAK416B
- Fluorescence Standard 100 μL
  (2 mM in DMSO)
  Catalog Number MAK416C
- Exonuclease Positive Control 1 vial
  Catalog Number MAK416D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Corning® UV-Transparent 96-Well Half-Area Microplate (Catalog Number CLS3679)
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Sonicator (for bacterial culture)
- Water, Nuclease-free for Molecular Biology (Catalog Number W4502 or equivalent)
- Ammonium sulfate (Catalog Number A4418 or equivalent)
- BCA Protein Assay Kit- compatible with reducing agents (Biovision Catalog Number K818 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Keep Exonuclease Probe, Fluorescence Standard (2 mM in DMSO), and Exonuclease Positive Control on ice while in use.

Exonuclease Assay Buffer: Prior to use, warm to room temperature.

Exonuclease Probe: Reconstitute vial with 110 µL of Molecular Biology Grade Water. Aliquot and store at -20 ºC, protected from light. Avoid multiple freeze-thaw cycles.

Fluorescence Standard (2 mM in DMSO): Prior to use, warm to room temperature. Store at -20 °C.

Exonuclease Positive Control: Reconstitute vial with 100 µL of Exonuclease Assay Buffer. Aliquot and store at -20 ºC. Avoid multiple freeze-thaw cycles.

Saturated Ammonium Sulfate Solution: Prepare a 4.1 M solution of ammonium sulfate (not included) in purified water at 25 °C.

Procedure

Sample Preparation

Bacterial cell culture

1. Collect bacterial cells from 1 mL of culture by centrifugation at 4000 × *g* for 10 minutes.
2. Discard the supernatant and resuspend with 100 µL of Exonuclease Assay Buffer.
3. Lyse the bacterial cells with a sonicator.
4. Centrifuge the lysate at >10,000 × *g*, 4 °C for 10 minutes and collect the supernatant.

Mammalian cell culture

1. Collect 500,000-1,000,000 mammalian cells by centrifugation.
2. Resuspend the cell pellet in 100 µL of Exonuclease Assay Buffer and rapidly homogenize the solution with a Dounce homogenizer.
3. Centrifuge the lysate at >10,000 × *g*, 4 °C for 10 minutes and collect the supernatant.

Animal tissue

1. Rapidly homogenize 10 mg of tissue in 100 µL of Exonuclease Assay Buffer with a Dounce homogenizer.
2. Centrifuge the lysate at >10,000 × *g*, 4 °C for 10 minutes and collect the supernatant.
3. Add 2 volumes of saturated ammonium sulfate solution into the supernatant (200 µL $(NH_4)_2SO_4$ for 100 µL of Sample). Place on ice for 30 minutes to precipitate the protein.
4. Centrifuge at >10,000 × *g*, 4 °C for 10 minutes and collect the precipitate.
5. Resuspend the precipitated protein in 100 µL of Exonuclease Assay Buffer. Pipette up and down to make sure that the precipitated protein completely dissolves.

For All Samples

1. Add 2-25 µL of the prepared sample in two parallel wells in the Half-Area Plate (not included) and label as Sample (S) and Sample Background Control (BC).
2. Adjust all Sample, Sample Background Control, and Positive Control wells to a total volume of 25 µL with Exonuclease Assay Buffer.

   For unknown samples, prepare several dilutions to make sure that the kinetic curve falls in the range of the Standard Curve.

   If expressing activity in µU/µg protein, determine the sample protein concentration. Exonuclease Assay Buffer contains DTT, so use of a BCA protein assay kit compatible with reducing agents is required.

Positive Control

Add 5-10 µL of the Exonuclease Positive Control into a well in the Half-Area Plate (not included).

Standard Curve Preparation

1. Prepare a 100 µM Standard solution by diluting 50 µL of the 2 mM Fluorescence Standard with 950 µL of purified water.
2. Prepare a 5 µM Standard solution by adding 10 µL of the 100 µM Standard solution from Step 1 into 190 µL of purified water. Prepare Standards according to Table 1. Mix well.

Table 1.
Preparation of Fluorescence Standards

Well	5 µM Fluorescent Standard	Exonuclease Assay Buffer	Fluorescent Std (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	10
3	4 µL	46 µL	20
4	6 µL	44 µL	30
5	8 µL	42 µL	40
6	10 µL	40 µL	50

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. Reaction Mix is added into Sample (S) and Positive Control wells. For each well, prepare 25 µL of Reaction Mix according to Table 2, mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix
Exonuclease Probe	1 µL
Molecular Biology Grade Water	24 µL

2. Add 25 µL of the Reaction Mix to each well containing the Sample (S) and Positive Control. Add 25 µL of Molecular Biology Grade Water into the wells containing Sample Background Control (BC). Mix wells for 30-60 seconds.

Measurement

Measure the fluorescence (λ_{Ex} = 304 nm/λ_{Em} = 369 nm) in kinetic mode every 30 seconds for 30-60 minutes at 37 °C. Adjust GAIN/PMT setting of the fluorometer as necessary so that the Standard Curve readings are linear within the detection range of the instrument.

Results

1. Subtract the 0 pmol Standard reading from all Standard readings.
2. Plot the Fluorescence Standard Curve with pmol of DNA on the x-axis and RFU on the y-axis.
3. Apply a linear fit to the Fluorescence Standard values and determine the Standard Curve equation.
4. Plot changes in RFU for each Sample on the y-axis vs. time (in minutes) on the x-axis and determine the slope (RFU/min) of the linear portion of the reaction curve.
5. Apply (RFU/min) to the Fluorescence Standard Curve to obtain the activity (A) of the Samples (pmol/min).
6. Subtract Sample Background readings (A_{BC}) from all Samples readings (A_S) to obtain the corrected activity (A_C):

$$A_C = A_S - A_{BC}$$

Exonuclease Activity (pmol/min/mL or µU/µL) =

$$(A_C/V) \times D$$

Specific Activity (pmol/min/µg or µU/µg) =

$$(A_C/[V \times P]) \times D$$

where

V = Volume of sample added in the reaction well (in µL)
D = Dilution factor
P = Protein concentration (µg /µL)
A_C = pmol/min (from the linear range of the activity curve)

Unit Definition: One unit of Exonuclease activity is the amount of enzyme that can digest 1 µmol of DNA molecule per minute at 37 °C.

Figure 1.
Typical Fluorescence Standard Curve

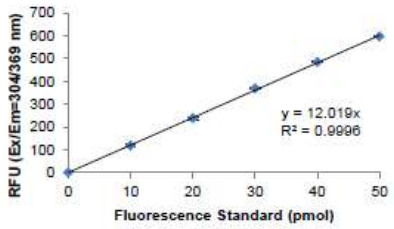


Figure 2.
Specificity of the probe to 3′ to 5′ exonuclease (Exonuclease III) in contrast to DNase I and Exonuclease I. Same units of proteins were added as defined by the commercially available values.

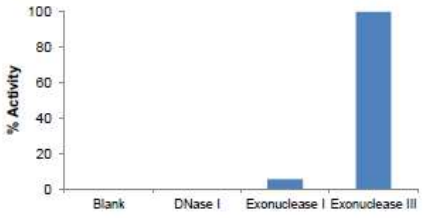


Figure 3.
Kinetic curves of Rat Liver Extract (40.6 μg), HeLa Cell Lysate (12.2 μg) and *E. coli* crude extract (0.642 μg).

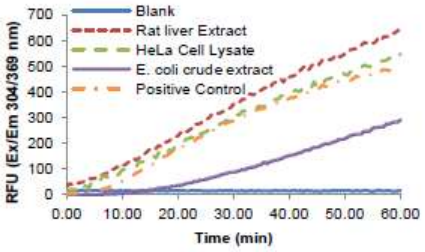
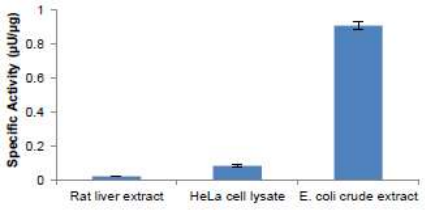


Figure 4.
Specific 3′ to 5′ exonuclease activity from Rat Liver Extract (0.0206 μU/μg), HeLa Cell Lysate (0.0841 μU/μg) and *E. coli* crude extract (0.907 μU/μg). Assays were performed according to the kit protocol.



Frequently Asked Questions

Does this kit measure exonuclease activity of single stranded DNA or double stranded DNA?

DNA probe used in this kit is a single stranded DNA probe. Hence this kit measures the exonuclease activity of single stranded DNA.

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